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Cue-conflict experiments between magnetic and visual cues in dunlin Calidris alpina and curlew sandpiper Calidris ferruginea

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Response to Reviewers:	Dear Dr Bakker, Thank you for your positive decision on the manuscript No. BEAS-D-16-00614R1 "Cue-conflict experiments between magnetic and visual cues in dunlin Calidris alpina and curlew sandpiper Calidris ferruginea". I checked all comments and made the requested corrections. I inserted the requested p values for the t-tests in the Results section (lines 194 and 211); however I can not use the exact p value for the Moore test (lines 195 and 204) since the values derive from the table in the paper by Moore (1980; Biometrika 67:175–180).

	Best Regards, Lorenzo

Cue-conflict experiments between magnetic and visual cues in dunlin *Calidris alpina* and curlew sandpiper *Calidris ferruginea*

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Abstract

Despite our extensive knowledge on various aspects of their lives, there has been limited investigation into the hierarchical relationships among different compass systems in shorebirds. The aim of this study was to investigate the relationship between magnetic and celestial compasses in two species of shorebirds, the curlew sandpiper (*Calidris ferruginea*; pre-breeding migration) and the dunlin (*Calidris alpina*; post-breeding migration) using cue-conflict experiments. Birds were captured in a Mediterranean stopover site, after which their magnetic orientation was determined under simulated overcast conditions at sunset using modified Emlen funnels fitted with infrared video cameras. Birds that demonstrated a well-defined directional preference were then exposed over two sunsets to conflicting directional information between the local geomagnetic field and the ± 90° shifted band of maximum polarisation. These individuals were tested again for magnetic orientation at sunset in the same conditions as previous test, to determine whether their directional choices had changed after the cue-conflict. Our results showed that individuals from both species did not recalibrate their magnetic compass from visual cues after the cue-conflict, even though at least dunlins did not appear to completely disregard the information derived from celestial cues. This study is one of the few experimental studies on the migratory orientation of *Charadriiformes* and on the hierarchical relationships between the different compasses used by these birds during their extensive migratory movements.

Keywords: *orientation, cue-conflict, polarized light, shorebirds, magnetic compass*

Significance Statement

Migrating birds are able to use different compass mechanisms based on geomagnetic or celestial cues and it seems reasonable to hypothesise that birds calibrate their various compasses to maintain the correct direction especially when

the directional information does not agree. The hierarchy among different compasses has been studied largely on night migrating passerines, but it is still poorly understood. We investigated the hierarchy among geomagnetic and celestial cues (band of maximum polarization) in two species of *Charadriiformes* by means of cue-conflict experiments. Our result showed that the geomagnetic cues have a dominant role in the orientation mechanisms of the studied species, even though the information derived from celestial cues did not appear to be completely disregarded.

Introduction

Migrating birds are able to use different compass mechanisms based on geomagnetic (Wiltschko and Wiltschko 1996) or celestial cues, including the sun (Kramer 1953; Moore 1987), stars (Emlen 1975) and skylight polarization pattern (Able and Able 1995; Cochran et al. 2004; Muheim et al. 2006b). However, the redundancy of various cues may be a problem for migrating birds, especially when the directional information does not agree (Wiltschko and Wiltschko 1999; Muheim et al. 2006a). Therefore, it seems reasonable to hypothesise that birds calibrate their various compasses to maintain the correct direction (Muheim et al. 2006a; Sjöberg and Muheim 2016), however, the hierarchy among different compasses remains poorly understood (Liu and Chernetsov 2012). While there is general agreement that celestial cues dominate over magnetic cues during the pre-migratory period (Bingman 1983; Able and Able 1990; Prinz and Wiltschko 1992; Wiltschko et al. 1998; Wiltschko and Wiltschko 1999), the relationship between these cues during migration remains unclear (Muheim et al. 2006a; Wiltschko and Wiltschko 2009). It has recently been proposed that at sunset, and possibly also at sunrise, the Band of Maximum Polarization of skylight (BMP) may be the reference system used by birds to recalibrate their magnetic compass during the migratory period (Muheim et al. 2006a, b). This use of BMP would give birds a reference system that is independent from both topography (Liu and Chernetsov 2012) and magnetic anomalies. Unlike the sun compass (Schmidt-Koenig 1990), the use of BMP does not require any time compensation as at sunrise and sunset it intersects vertically the horizon roughly along the North-South axis, independent of latitude (Phillips and Waldvogel 1988; Muheim et al. 2006a). Some cue-conflict (CC) experiments seem to have confirmed this hypothesis (Phillips and Moore 1992; Cochran et al. 2004; Muheim et al. 2006b, 2007), however, the role of polarisation pattern is still a topic of debate (Wiltschko et al. 2008a, b; Muheim et al. 2008). Further studies on both caged and free-flying birds have cast doubts on the use of BMP for recalibrating magnetic references (Chernetsov et al. 2011; Schmaljohann et al. 2013; Åkesson et al. 2015), or have suggested that birds may not always reconcile conflicting information from geomagnetic and celestial (star) cues, therefore making a form a compromise between the directions indicated by the two reference systems (Giunchi et al. 2015). Sjöberg and Muheim (2016) recently attempted to integrate the different outcomes by suggesting that the discrepancies observed between

cue-conflict experiments may be due to a combination of both the availability of polarised light information near the horizon and stars. While intriguing, this explanation is highly speculative and a number of experimental observations remain unexplained (see Sjöberg and Muheim 2016 for further details).

Until recently, almost all available data concerning the orientation mechanisms of wild birds have been derived from experiments on a few species of nocturnal passerine migrants. Furthermore, while there is a significant amount of information regarding the migratory ecology and routes of shorebirds (see Colwell 2010), only few studies have investigated the orientation mechanisms of these species. To our knowledge, only four papers have been published on this topic. Sauer (1963) and Sandberg and Gudmundsson (1996) initially investigated shorebird orientation in modified Emlen funnels (Emlen and Emlen 1966), demonstrating that both Pacific golden plovers (*Pluvialis fulva*) and dunlins (*Calidris alpina*) showed directional preferences consistent with the predicted migratory direction when tested under a clear sky. Following these initial observations, the use of magnetic cues were demonstrated by studies that showed that sanderlings (*Calidris alba*; Gudmundsson and Sandberg 2000) and sharp-tailed sandpipers (*Calidris acuminata*; Grönroos et al. 2010) responded as expected to a ± 90° deflection in the local magnetic field. To date, the hierarchy between different compass mechanisms has not yet been investigated in shorebirds.

The aim of the present study was to investigate the relationship between magnetic and visual compasses (i.e. the pattern of skylight polarisation) in two species of shorebirds using cue-conflict experiments.

Materials and methods

Study location and experimental birds

Birds were caught with mist nets during the pre-breeding (curlew sandpiper *Calidris ferruginea*; April–May) and post-breeding (dunlin *Calidris alpina*; August–September) migrations from 2012 to 2015 in the Lame di San Rossore wetland complex (Massaciuccoli Regional Park, Migliarino, San Rossore, Pisa, Lucca, Italy; 43°41'N, 10°17'E). After the standard ringing procedures (Busse and Meissner 2015) the birds were aged and sexed according to Prater et al. (1977). Due to the migratory strategies of these species (del Hoyo et al. 1996), our experimental sample of curlew sandpiper was composed entirely of adults, while we tested both adults and juvenile dunlins. Birds were weighed using an electronic scale (± 0.1 g) and then held in captivity in the Arnino Field Station (43°39'N 10°18'E; capture site direction = 327°, capture site distance = 3.5 km) for a maximum of 10 days in cylindrical plastic cages (diameter = 100 cm, height = 30 cm). The birds were fed with mealworm (*Tenebrio molitor*) larvae and were provided water *ad libitum*. According to the social habits of the considered species (del Hoyo et al. 1996), individual were kept in groups of 3 to 5 birds per cage. The cages were placed in rooms with artificial lighting that was synchronised with the natural

photoperiod so that birds could not observe any celestial cues. During captivity, the mean weight of the curlew sandpipers and dunlins increased by 9.7 g ($t_{12} = 6.7$, $p < 0.01$, paired t-test; capture weight: 49.7 ± 3.6 g SD) and 13.8 g ($t_9 = 10.4$, $p < 0.01$, paired t-test; capture weight: 39.5 ± 2.7 g), respectively.

Experimental procedures

Our experimental protocol was followed as described below:

1) After a minimum of two days in captivity the magnetic orientation of birds was tested at sunset under simulated overcast conditions (hereafter referred to as the pre-CC test).

2) Birds that demonstrated a well-defined directional preference in the pre-CC test were exposed (CC exposure) at sunset for two consecutive days to conflicting directional information between the local geomagnetic field and the ± 90° shifted BMP (for further details, see Giunchi et al. 2015). After each CC exposure, birds were returned to their indoor cages and prevented from seeing any visual cue.

3) Treated birds were tested again the day following the second CC exposure for their magnetic orientation at sunset in the same conditions as the pre-CC test. The purpose of this test (post-CC test) was to evaluate the consistency of directional choices exhibited before and after the cue-conflict.

Experimental apparatus

The orientation tests were performed using modified Emlen funnels which were made out of non-magnetic transparent material (LEXAN; Sabic Innovative Plastic, Pittsfield, MA), the size of which was consistent with that described by Sandberg and Gudmundsson (1996) (top external diameter = 52 cm, base diameter = 18 cm and height = 22 cm). Each funnel was divided into eight sectors of 45° using strips of tape, and then placed in plastic containers (70 cm high). An infrared camera was positioned at the bottom of the plastic containers (see Online Resources, Fig. S1) which was powered by a 12 V battery and connected to a digital video recorder. Each funnel was covered by plastic mesh netting (2 × 2 mm). We measured the magnetic field inside the funnels using a fluxgate magnetometer (Applied Physics Systems, Mountain View, CA) to ensure that the magnetic field perceived by the experimental birds was not affected by the experimental apparatus. Before the beginning of each test, each funnel was oriented randomly with respect to the magnetic North. A cubic wooden box (90 × 90 × 50 cm) with four square windows (48 × 48 cm) was placed on the top of each funnel in the centre of each side. The top of the funnel and the windows were covered by milky-white Plexiglass lids (3 mm thickness). This structure prevented birds from perceiving the light polarisation (Sandberg et al. 1988; Åkesson et al. 2015) and other visual cues while also avoiding excessive darkness inside the funnels (Fig. S1).

To manipulate the skylight polarisation pattern we replaced the Plexiglass windows on the same cubic wooden boxes with panels of two outer layers of pseudo-depolarising filters composed of two sheets of polyester (180 μm thick; Metalloy Italiana, Vicenza, Italy) which were aligned at an angle of 45° relative to each other, in addition to one inner layer composed of a polarising filter (0.8 mm thick, cellulose triacetate; Intercast Europe, Parma, Italy), as described by Gaggini et al. (2010). The polarising filters from the two opposite windows were aligned so that the e-vector was vertical, while the e-vector was horizontal for the other two windows (Gaggini et al. 2010). The top of each box was covered by a milky-white Plexiglass lid (3 mm thick).

During CC exposure, the birds were kept in a small wooden cage (40 × 20 cm) with netting on each side, which was placed in the centre of the larger wooden box. The small size of this holding cage compared to the box was used to reduce any parallax effect on the alignment of the artificial polarisation axis (Muheim et al. 2006b). When the vertical filters were aligned with the solar azimuth, the caged birds perceived the BMP shifted by ± 90° relative to natural conditions (Gaggini et al. 2010). The entire structure was placed on wooden stands (70 cm high) to give the birds a broad view of the horizon through the polarising filters.

We performed both orientation tests and exposures to cue-conflict only in calm evenings with a wind speed of < 10 m/s and no rain. We did not perform exposures to cue-conflict when cloud cover was higher than 3/8. The orientation tests lasted for 40 minutes and were performed between 20 minutes before and 70 minutes after the civil twilight (sun elevation < -6°). Exposures lasted for 40 minutes and occurred between 20 minutes before and 20 minutes after civil twilight. The orientation of each individual was tested over a maximum of three consecutive evenings, and birds that remained inactive (see below) during all these trials were released close to the capture site. The first test in which the individual showed to be active was considered for the analyses.

Data analyses

The video recordings were analysed to determine the directional preferences of each individual. The first 10 minutes of the recordings were discarded in order to allow the birds to acclimatise to the funnel. The remaining 30 minutes were subdivided into 600 frames (1 frame every 3 seconds) which was then combined into a single multipage Tiff file. A circle was drawn on each image 6 cm from the bottom of the funnel for use as the reference line. Only well-defined movements (WDMs) were considered for the analysis, i.e. only when birds crossed the reference line with at least a third of the front of its body. The WDMs within a given sector were assigned to the mid-angle of the sector. Only active birds (WDMs ≥ 10) were included in the analyses. Individual directional preferences were calculated as the circular mean of the WDM distribution (Batschelet 1981). Each of the multipage Tiff files was independently examined by three

observers blind to the experimental condition. As the circular means estimated by the three observers did not differ by more than 30°, all active birds were included in the analysis. The orientation of each bird was calculated by averaging the three directional choices estimated by the three observers. Birds were excluded from the analyses if the result of the Rayleigh test (Batschelet 1981) applied to the circular distribution of their individual headings was not significant ($p > 0.05$) . As previously reported (see e.g. Muheim and Åkesson 2002), the Rayleigh test was only used as a guideline as the movement of birds inside the funnel could not be considered to be independent. Actually all active birds of both species turned out to be oriented according to this criterion.

The group statistics were calculated from individual mean directions so that each data point represented one bird (Batschelet 1981). When the mean vector length resulting from doubling the angles was larger than the unimodal vector length, we used a mean axis of orientation as the basis of the analysis. Randomness was tested with the Rayleigh test and the 95% confidence interval for the mean group directions were calculated using bootstrap methods with 5000 resampled datasets (Adams and Anthony 1996) only for significantly oriented samples. The effect of CC exposure was determined by analysing the distribution of the angular differences of individual headings between the pre-CC and post-CC tests (post–pre) and using the non-parametric paired-sample test described by Moore (1980). As either unimodal and axially bimodal circular distributions did not seems to describe satisfactorily the post-pre sample distribution in dunlins, we used a model based clustering approach (Hornik and Grün 2014 and references therein) by means of the R package Directional 2.4 (Tsagris et al 2016) to evaluate whether a mixture of two von Mises-Fisher distributions fitted the data better than the standard unimodal model. We firstly calculated the Bayesian information criterion (BIC; Schwarz 1978) of the models with one or two components using the bic.mixvmf function, assuming a mixture of two von Mises-Fisher distributions. The model with the lowest BIC value was chosen to best describe the data. If a bimodal model had the lowest BIC value then a mixture of two von Mises-Fisher distributions were fitted to the data using the mix.vmf function, which gave the predicted group assignment for each observation. As a check of this approach, we applied this method to all samples (Table S1). All statistical analyses were performed with the software R 3.3.2 (R Core Team 2016); standard circular statistic analyses were performed using the R package circular 0.4–7 (Agostinelli and Lund 2013).

Expectations

We expected that in the pre-CC test the birds would orient themselves according to the distribution of recovery/recapture of birds ringed in Italy during the spring (curlew sandpiper) or autumn (dunlin) migration (Spina and

Volponi 2008). Depending on the hierarchy determined by the information provided by the celestial cue (BMP) and the geomagnetic field, each individuals could respond to the cue-conflict in two possible ways:

If the magnetic cue was dominant over BMP, we expected that there would be no differences in the orientation of the birds between the pre-CC and post-CC tests (Fig. 1).

If BMP was used to calibrate the magnetic compass, we expected to observe a significant ± 90° shift in directional preferences in the post-CC test compared to the pre-CC test (Fig. 1; Cochran et al. 2004; Muheim et al. 2006b).

Results

Curlew sandpiper

30 adult curlew sandpipers tested in the spring of 2013 and 2014, 15 were active in the pre-CC test. Two birds were inactive during the post-CC test for three consecutive trials, therefore, they were excluded from the analyses. The directional preferences of the remaining 13 individuals were bimodally distributed during the pre-CC test along the WSW-ENE axis (Fig. 2). We did not find any difference in body weight between WSW [225°-300°] and ENE [350°-110°] oriented birds (54.5 ± 4.3 g vs 57.4 ± 3.2 g; $t_{10.8} = 1.37$, $p = 0.2$, Welch's t-test). The distributions before (pre-CC test) and after (post-CC test; see Fig. 2) the CC exposure were not significantly different ($r_m = 0.12$, $p > 0.05$, Moore test). The angular differences between the post-CC test and the pre-CC test (post–pre) were unimodally distributed and the mean direction ($\alpha = 13°$) was not significantly different from 0° (95% CI = [331°-35°]; Fig. 2).

Dunlin

We tested 19 dunlins during the post-breeding migration between 2012 and 2015. Eleven birds were active in the pre-CC test, but only 10 (five juveniles and five adults) were found to be active in the post-CC test and hence included in the analyses. In the pre-CC test (Fig. 2) birds headed in a SSW direction, and no significant differences were recorded between the heading distributions before (pre-CC test) and after (post-CC test; see Fig. 2) the *CC* exposure ($r_m = 0.70$, $p > 0.05$, Moore test). The angular differences between post-CC test and pre-CC test (post–pre, Fig 2) were unimodally distributed with a mean direction of – 58° (95% CI: [261°-342°]). The model based clustering approach analysis indicated that a model with two components (a mixture of two von Mises-Fisher distribution) described the post-pre distribution better than a unimodal model (BIC = 33.07 vs 33.47, respectively; see Table S1). This approach identified two well-defined clusters: birds belonging to one cluster shifted their directional preferences 109° counter-clockwise,

while individuals belonging to the second cluster did not change their directional preferences after the cue conflict (Fig. 2; Table S1). The mean weight of birds belonging to the two cluster, recorded just before the post-CC test, was not significantly different (cluster 1 = 52.5 ± 7.2 g vs cluster 2 = 54.1 ± 0.7 g; $t_{4.08} = 0.52$, $p = 0.63$, Welch's t-test)

Discussion

This work is one of the few studies aimed at investigating the migratory orientation of *Charadriiformes* and the hierarchical relationship between the visual and magnetic compass systems. Our results show that both curlew sandpipers and dunlins are able to orient themselves under simulated overcast conditions, as previously demonstrated for sanderlings (Gudmundsson and Sandberg 2000) and sharp-tailed sandpipers (Grönroos et al. 2010).

The percentage of inactive individuals was noticeable (ca. 50% in both species), but it is consistent with the results of a number of orientation studies on waders, which reported that these birds are more likely to not show migratory restlessness when tested under overcast conditions (see e.g. Sandberg and Gudmundsson 1996; Gudmundsson and Sandberg 2000, but see Grönroos et al. 2010). Even though free-flying waders can depart from stopover/staging sites and orient correctly under solid overcast, it should be noted that (1) Alerstam et al. (1990) reported that in most occasions knot and turnstone flocks departed under clear sky conditions and (2) all the 277 departures of wader flocks observed by Piersma et al. (1990) in the Dutch Wadden Sea occurred when the sun was clearly visible. Furthermore, it should be noted that the migratory behaviour of shorebirds is often quite different from that of passerines, being characterized by long flights followed by prolonged stay at staging site (Piersma 1987); this behaviour might affect the motivation to migrate of birds captured while refuelling thus increasing the inter-individual variability in the expression of migratory restlessness. For this reasons we suggest that the fraction of inactive birds is not an artifact due to our experimental apparatus, but a consequences of having tested birds without available visual cues.

Orientation preferences in the pre-CC test

Dunlins showed a clear directional preference toward a SSW direction, which is in agreement with the distribution of the ringing recoveries reported by Spina and Volponi (2008) and to the expected direction for the post-breeding migration (Cramp and Simmons 1983). The bimodal distribution of the directional preferences of curlew sandpipers is more puzzling. According to previous studies (Wilson et al. 1980; Delany et al. 2009), curlew sandpipers crossing the Mediterranean appear to follow an eastern route during spring migration, moving from Western Africa to their breeding grounds via Tunisia, Crimea and the Caspian Sea. The number of Italian recoveries of abroad ringed birds during the

spring migration is quite low; however, most of the recoveries were located along a SW-NE axis between the African Atlantic coast and the Crimea peninsula (Scebba and Moschetti 2006; Spina and Volponi 2008). Therefore, the axis of orientation of tested curlew sandpipers is consistent with the migration axis of migrating Curlew sandpipers in spring, as supported by Italian ringing data. The fact that the majority of birds preferred a westward direction is however problematic. The orientation of experimental birds may have been affected by a positive phototaxis towards the setting sun (see e.g. Åkesson and Sandberg 1994; Muheim et al. 2006a), as the solar azimuth in the mid-period of the experiments was approximately 295°. This explanation seems however unlikely because the experiments were performed under simulated overcast conditions obtained by opaque milky plexiglass lids, which diffuse the low ambient light present at the time of the experiments. A phototactic response should characterize mostly birds tested in the first period of the experiments (20 minutes before to 20 minutes after the civil twilight), but this was not confirmed by the data reported in Fig. 2 (pre- and post-CC tests). Furthermore, this kind of response was absent in dunlins, that were all tested in the first period of the experiments (sun azimuth in the mid-period of the experiments = ~287°). The directional preferences of the sub-group westward oriented can be interpreted as reverse migration (Åkesson et al. 1996). While reverse migration is more likely when the access to visual cues is prevented (Åkesson et al. 2001; Nilsson and Sjöberg 2016), such as in this study, this behaviour is usually shown by lean birds facing an ecological barrier (Sandberg and Moore 1996; Åkesson et al. 1996; Sandberg et al. 2002; Deutschlander and Muheim 2009; Schmaljohann et al. 2011). The individuals tested were found to be in good condition (see Materials and Methods) and did not appear to be facing an ecological barrier, therefore, this explanation seems unlikely. It can be speculated that at least a part of the tested individuals oriented toward the sea-coast (located about 1.7 km west of the test site), possibly relying on infra-sounds, as previously suggested for homing pigeons (Hagstrum 2000, 2013). In addition to providing an important food source (Colwell 2010), the coastline is also an important landmark for migrating birds (Åkesson 1993), and therefore, the directional preferences of the curlew sandpipers may have been affected by the closeness of the coast. However, it should be noted that this effects was absent in dunlins; moreover, as reported by Holland (2014) the effect of infrasound on bird migration has never been experimentally demonstrated.

Response to the cue-conflict

We did not record any significant difference between pre- and post-CC distributions in both species, which suggest that neither dunlins nor curlew sandpipers significantly changed their directional preferences after CC exposure, contrary to that expected if birds had recalibrated their magnetic compass based on the artificial skylight polarisation pattern (Cochran et al. 2004; Muheim et al. 2006a, b, 2007, 2009). Our results are hence in broad agreement with recent studies

indicating the primary role of the magnetic cues in the migratory orientation of passerines (Gaggini et al. 2010; Chernetsov et al. 2011; Schmaljohann et al. 2013; Åkesson et al. 2015). Actually, the majority of songbird species tested did not appear to recalibrate their magnetic compass based on the skylight polarisation pattern (Åkesson et al. 2015). The results from the current study add two species of non-passerine long-distance migrants to this pattern, and taken together, these results suggest that recalibration is less widespread than that hypothesized by Muheim et al. (2006a). However, the outcomes from some studies may have been affected by issues in methodology (see also Giunchi et al. 2015). According to Wiltschko et al. (2008a) the experimental protocol also used in our experiments may lead to artefacts due to the totally artificial nature of the band of polarization. It should to be noted that the experiments performed by Muheim et al. (2009), where birds were exposed either to shifted magnetic fields or to shifted artificial polarization pattern, produced consistent results, thus suggesting that the potential artefacts due to polarizing filters *per se* are at least negligible. Sjöberg and Muheim (2016) recently modified the model proposed by Muheim et al. (2006a) to suggest that the discrepancies observed in the various experiments may be due to a combination of the availability of polarised light information near the horizon and of the stars during the conflict. According to this model, birds recalibrate their magnetic compass using polarised light cues, provided they have access to the BMP and the view of surroundings. In our study birds had a full view of the BMP and distant landmarks (a pinewood strip running parallel to the coast, in particular). Therefore, according to Sjöberg and Muheim (2016), we would still expect to observe recalibration of the magnetic compass even though the birds did not have access to stars information. This recalibration however was not observed, as no significant difference between pre- and post-CC tests was recorded for both species. Birds might not had recalibrated their magnetic compass because of a "switching off" of the calibration after prolonged exposure to visual landmarks in the same area (Sjöberg and Muheim 2016). Our tested birds were displaced from the capture site and held indoor with no access to landmarks before and during the experiments; they could see the surroundings only during the exposure to the cue-conflict. It thus seems quite unlikely that they did not pay attention to calibration cues due to the familiarity to local landmarks. It can be suggested that birds did no recalibrate their magnetic compass because they could rely on infra-sounds to sense the sea-coast which can be used as a prominent directional cue. While deserving further investigation, this explanation has to be regarded as highly speculative, as no experiment has demonstrated yet the effect of infra-sounds on the orientation of migratory birds (Holland 2014). The reaction of birds to the cue-conflict might depend also on the reliability of the compass information that they experienced in different geographic areas (Chernetsov et al. 2011; Åkesson et al. 2015). It should be noted, however, that the analysis reported by Sjöberg and Muheim (2016) did not find any relationship between the temporal and spatial variability of

magnetic field properties (declination, intensity and inclination) and the outcomes from cue-conflict experiments performed in North America and Europe.

While the heading distributions obtained before and after the CC exposure was not significantly different in dunlins, the 95% CI of the mean direction of the angular differences between pre- and post-CC tests did not include 0°, while they marginally included 90°, thus indicating that dunlins shifted their orientation after the CC exposure at least slightly. Actually, according to the model-based clustering approach, the circular distribution of these angular differences should be properly described as a mixture of two unimodal distributions, which suggests that the way dunlins reacted to the cue-conflict was individually variable, but that the skylight polarisation pattern is not completely disregarded. According to this model, half of the tested dunlins (cluster 1 in Fig. 2) did not change their directional preferences after the cue-conflict, whereas the remaining individuals (cluster 2 in Fig. 2) shifted their directional preferences close to the 90° shift predicted if birds were to recalibrate their magnetic compass based on the skylight polarisation pattern. It can be speculated that this pattern might be due to the inclusion of birds in different phases of migration (see e.g. Wiltschko et al. 2008a), but this seems unlikely as dunlins were tested well after the beginning of their migration (Cramp and Simmons 1983). The heterogeneity observed in the responses to the cue-conflict might be ascribe also to population specific differences (i.e. birds breeding at different latitudes with different reliability of magnetic cues - Muheim et al. 2003; Åkesson and Bianco 2015) or to the previous experience of tested individuals, as the ecological context and the regional availability of orientation cues may affect the use of various compasses (Sandberg and Moore 1996; Chernetsov et al. 2011; Giunchi et al. 2015). Interestingly, the same pattern was not evident in curlew sandpipers, although even in this species the individual variability was not negligible. While we acknowledge that the sample size was small, these inter-specific differences may suggest an effect of different migratory periods (but see Chernetsov et al. 2011), even though possible inter-specific differences in the way the different cues are integrated might be considered. In addition, we cannot excluded an effect of the proximity of the coastline, as this could have also affected the directional preferences of curlew sandpipers during the post-CC test, which may have been responsible for the lack of an effect of the cue-conflict on the majority of tested birds.

In conclusion, the data reported in this study suggest that the variability observed in cue-conflict experiments may be affected by the responses of individuals in prioritising different cues. This individual variability should be taken into account when interpreting the results of cue-conflict studies, particularly with species that tend to live longer than small songbirds, and therefore, have more opportunities to learn from previous migrations.

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Ethical statement

Ethical approval: All protocols performed in studies involving animals complies with the ethical standards and Italian laws on animal welfare. All procedures involving animals were approved by the Italian Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA).

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Conflict of Interest: The authors declare that they have no conflict of interest.

Informed consent: Informed consent was obtained from all individual participants included in the study.

347

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Figure legends

Fig. 1 Scheme of the expectations from the cue-conflict experiments with curlew sandpipers, with an expected direction of north. The expectations for dunlins were similar, however, the expected direction was south instead of north. The direction of the magnetic north and geographic north are represented by orange and black triangles, respectively. See the Materials and Methods section for further details

Fig. 2 Heading distribution of curlew sandpipers and dunlins prior (pre-CC test) and after (post-CC test) the cue conflict and distribution of the angular differences in the heading of individuals between post-CC test and pre-CC test (post – pre). In curlew sandpipers, different colours were used to identify birds tested in the first (20 minutes before – 20 minutes after the civil twilight; light grey circles) or in the second experimental period (30 minutes – 70 minutes after the civil twilight; dark grey circles). Open and filled circles in dunlins were used to identify adults and juveniles, respectively. Cluster 1 and 2 correspond to the clusters identified by the model based clustering approach (see Materials and Methods and Table S1). The mean vector (α) of each distribution is represented by an arrow, the length (r) was drawn relative to the radius of the circle = 1. The significance is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and n.s. $p > 0.05$ as determined by the Rayleigh test. The 95% confidence intervals (broken lines) were reported only for significantly oriented samples

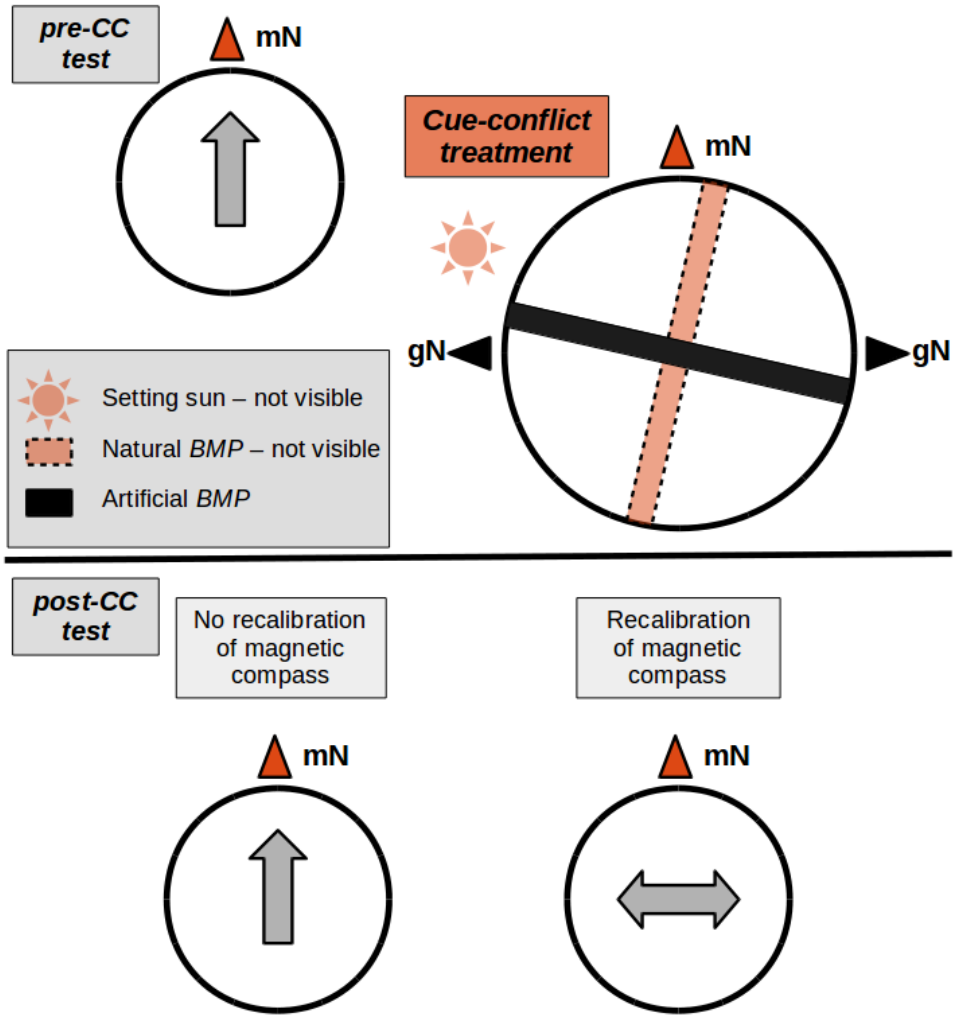
pre-CC test
mN
Cue-conflict treatment
mN
gN
gN
Setting sun – not visible
Natural BMP – not visible
Artificial BMP
post-CC test
No recalibration of magnetic compass
Recalibration of magnetic compass
mN
mN

Fig. 1

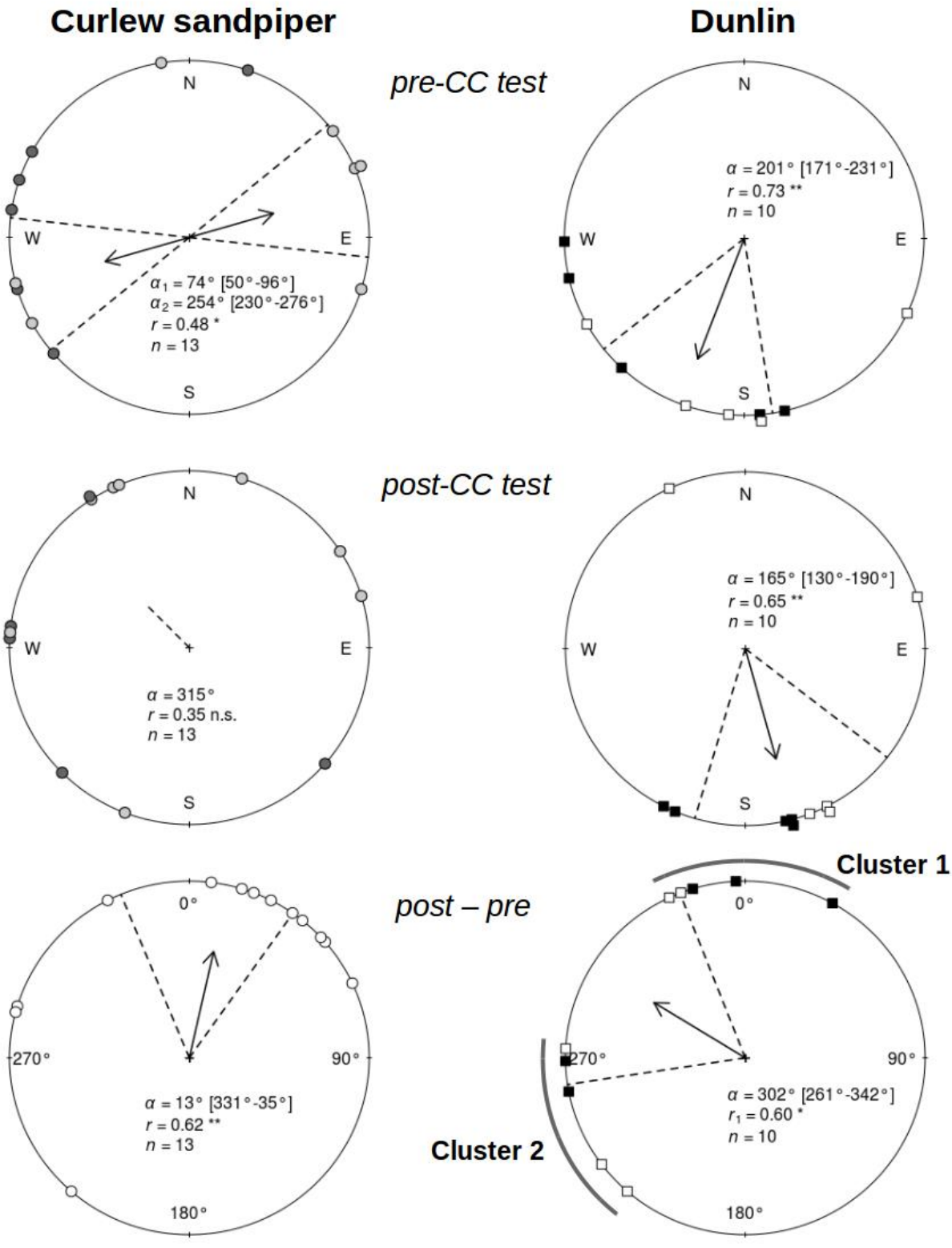
Curlew sandpiper
Dunlin
pre-CC test
N
W
E
S
α1 = 74° [50°-96°]
α2 = 254° [230°-276°]
r = 0.48 *
n = 13
α = 201° [171°-231°]
r = 0.73 **
n = 10
post-CC test
α = 315°
r = 0.35 n.s.
n = 13
α = 165° [130°-190°]
r = 0.65 **
n = 10
post – pre
0°
90°
180°
270°
α = 13° [331°-35°]
r = 0.62 **
n = 13
Cluster 1
Cluster 2
α = 302° [261°-342°]
r1 = 0.60 *
n = 10

Fig. 2

Figure

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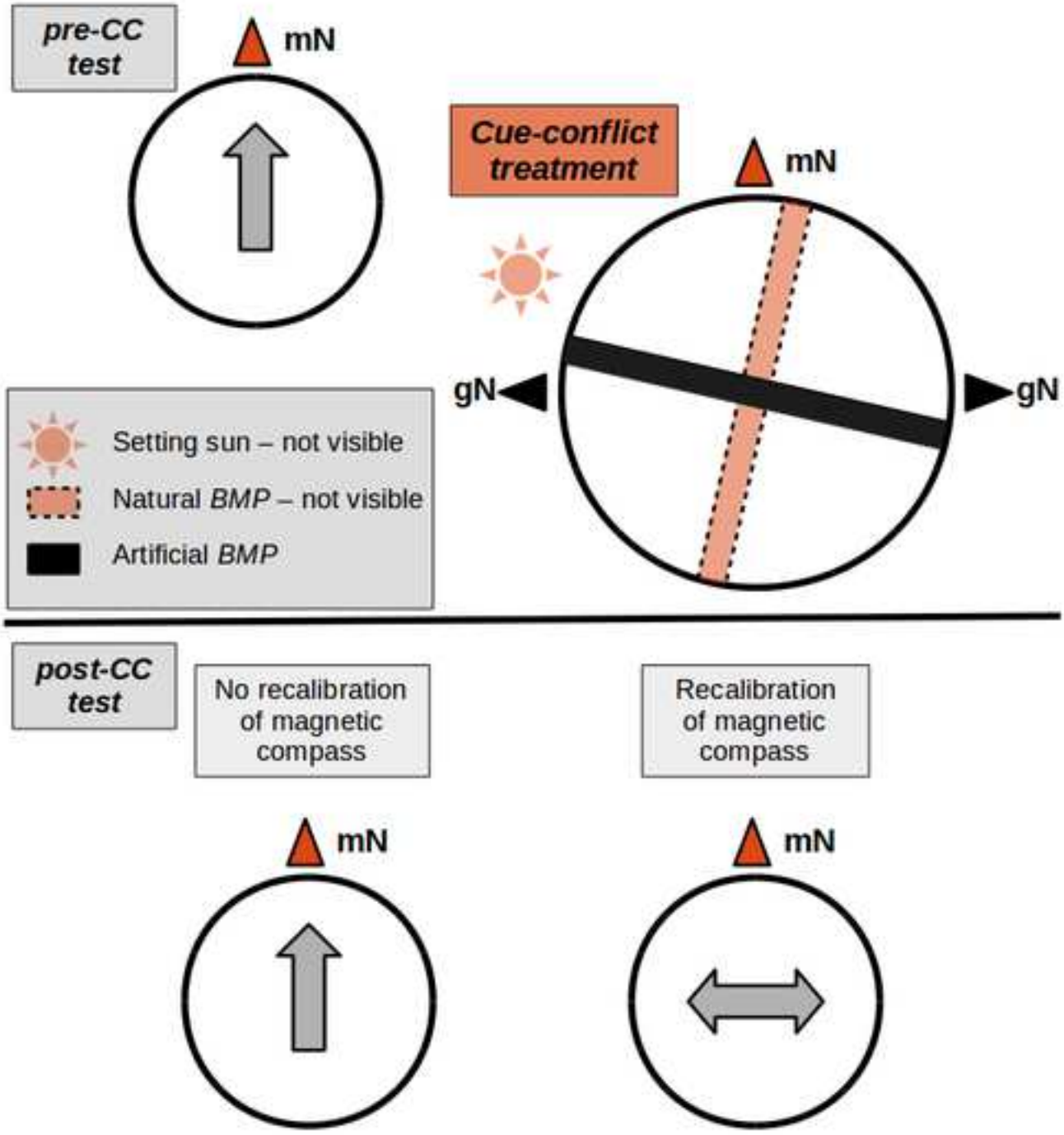

Figure

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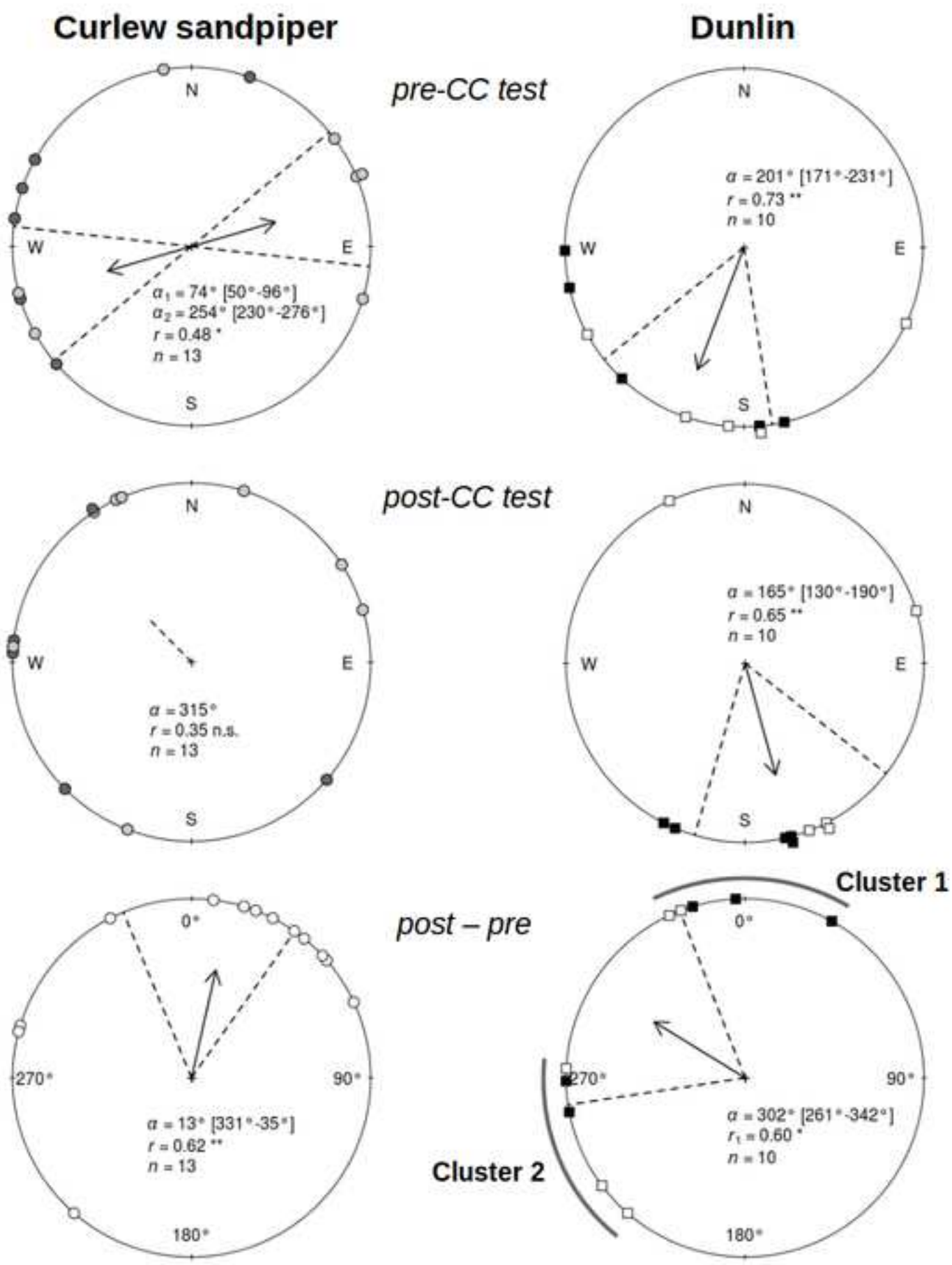

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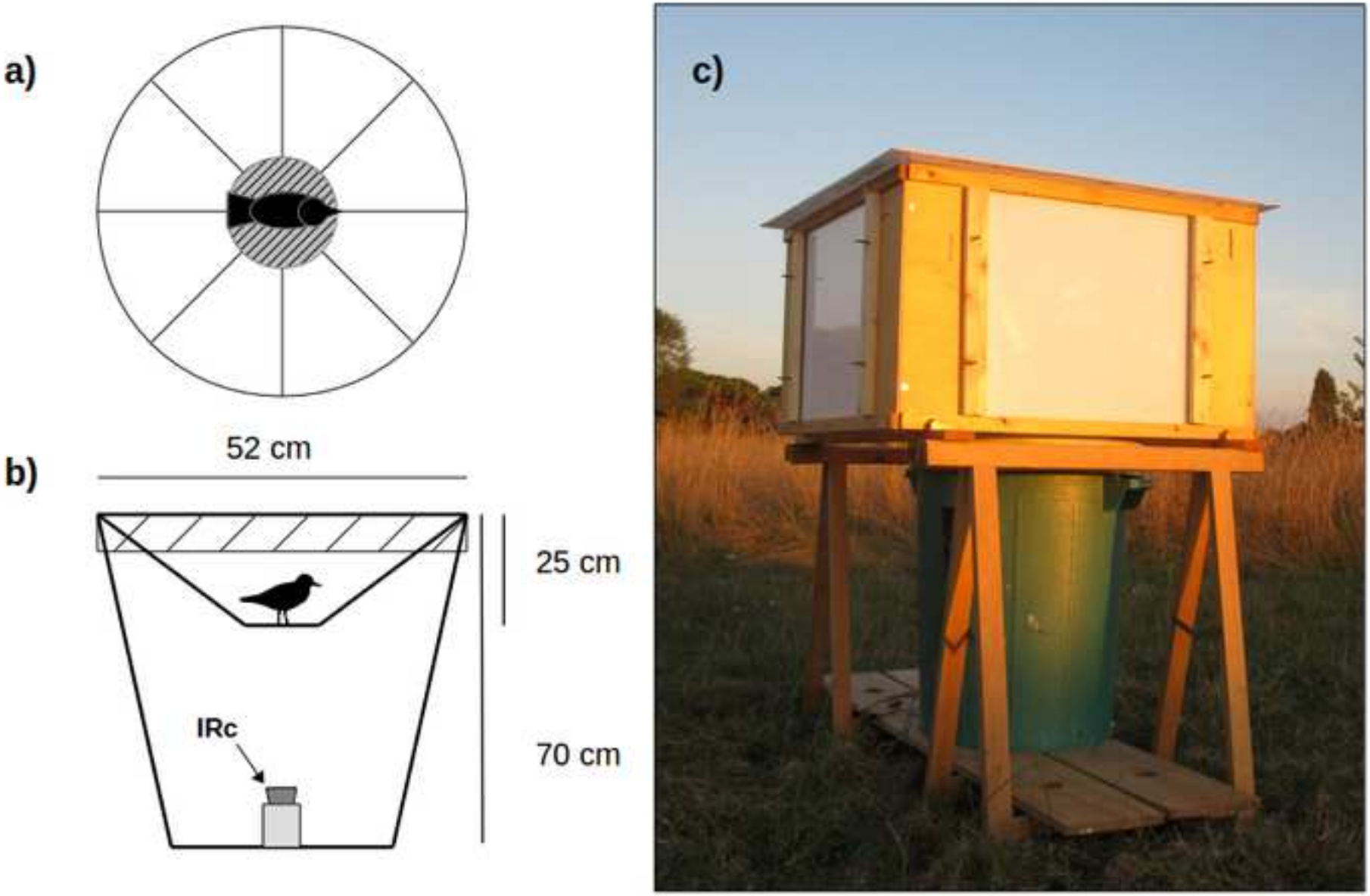

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